The Antimicrobial Peptide hLF1–11 Drives Monocyte-Dendritic Cell Differentiation toward Dendritic Cells That Promote Antifungal Responses and Enhance Th17 Polarization

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Key Words

Dendritic cell • Monocytes • Antimicrobial peptide •
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Abstract

The hLF1–11 peptide comprising the first 11 N-terminal residues of human lactoferrin exerts antimicrobial activity in vivo, enhances the inflammatory response of monocytes and directs monocyte-macrophage differentiation toward cells with enhanced antimicrobial properties. In this study, we investigated the effects of hLF1–11 on human monocyte-dendritic cell (DC) differentiation and subsequent T cell activation. Results revealed that – compared to control (peptide-incubated) DCs – hLF1–11-differentiated DCs displayed enhanced expression of HLA class II antigens and dectin-1, and increased phagocytosis of *Candida albicans*. In addition, hLF1–11-differentiated DCs produced enhanced amounts of reactive oxygen species, IL-6 and IL-10, but not IL-12p40 and TNF- α , upon stimulation with *C. albicans*. Moreover, 6-day-cultured hLF1–11-differentiated DCs and control (peptide-incubated) DCs that had been stimulated with a Th17-inducing mix of antigens (including *C. albicans*) for 24 h were co-cultured with autologous CD4⁺ T cells for 72 h and then the

levels of IL-10, IL-17 and IFN- γ production and the percentage of cytokine-producing T cells were assessed. The results revealed that the hLF1–11-differentiated DCs induced an enhanced IL-17, but reduced IFN- γ , production by T cells as compared to control (peptide-incubated) DCs. Collectively, the hLF1–11 peptide drives monocyte-DC differentiation toward DCs that promote antifungal responses and enhance Th17 polarization.

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Introduction

In tissues, dendritic cells (DCs) have the capacity to recognize and process pathogens and following their migration to the lymph nodes, present pathogen-derived antigens to T cells, thereby shaping an adaptive immune response. DCs can differentiate from a precursor cell depending on factors present in the local microenvironment. For example, when monocytes upon entering tissues encounter inflammatory mediators like cytokines, growth factors, chemokines, complement components and antimicrobial peptides (AMPs), they differentiate into immature DCs (iDC). These cells are known for

abundant expression of pathogen recognition receptors and HLA class II molecules, efficient antigen uptake and processing capacities. Upon tissue injury triggered by microbial agents or other danger signals, iDCs mature into antigen-presenting cells that produce inflammatory mediators, migrate toward lymphoid organs and instruct T lymphocytes to proliferate and differentiate into specific T cell subsets. This polarization of T cells depends greatly on the DCs and the inflammatory mediators, e.g. antimicrobial peptides, present in their environment.

AMPs are often cationic, relatively short and active against a variety of microorganisms including multidrug-resistant pathogens [1]. An increasing number of studies show the diversity in the mechanisms of action of these peptides, i.e. direct killing of pathogens [2, 3], modulation of pathogen development [4, 5] and modulation of immune cells [6–8] and were therefore also named as ‘host defense peptides’ (HDPs) [9, 10]. For possible therapeutic application of HDPs, it will be important to understand the interactions of these peptides with the host’s immune cells. For example, cathelicidin-based peptides IDR-1 [11] and IDR1002 [12] have been developed on the basis of their ability to enhance chemokine production by innate immune cells. The cathelicidin LL-37 is able to direct both monocyte-macrophage differentiation [13] as well as differentiation toward DCs that promote a Th1 response in vitro [14] through interaction with an intracellular target [15]. LL-37 is also able to modulate the adaptive immune response by directly affecting the maturation of DCs [16].

We recently reported that the antimicrobial peptide comprising the first 11 N-terminal residues of human lactoferrin, further referred to as hLF1–11, enhances the inflammatory response of monocytes and modulates monocyte-macrophage differentiation, an additional mechanism of action to its already established antimicrobial effects [3, 5, 17]. hLF1–11 enhances inflammatory mediator production by murine and human monocytes [18] and promotes GM-CSF-driven monocyte-macrophage differentiation toward a macrophage subset that shows enhanced responsiveness to microbial stimuli and demonstrates increased clearance of pathogens [6]. Since monocytes can also differentiate toward (immature) DCs, we have investigated the effects of hLF1–11 on monocyte-DC differentiation. We found that when hLF1–11 was present during GM-CSF/IL-4-driven differentiation of monocytes toward DCs, the resulting iDCs displayed enhanced antifungal responses against *C. albicans* and – upon maturation – enhanced IL-17 production while reducing IFN- γ production by T cells.

Materials and Methods

Peptides

The human lactoferrin-derived peptide hLF1–11 (GRRRRS-VQWCA; 1.374 kDa) was purchased from Peptisyntha (Torrance, Calif., USA) and the control peptide (GAARRAVQWAA; 1.115 kDa) from Isogen (De Meern, The Netherlands). The control peptide shows no activity against pathogens in vitro and in vivo [5]. The purity of both peptides was >97% as determined by reverse-phase high-performance liquid chromatography. Stocks of the peptides were made in phosphate-buffered saline (PBS, Department of Pharmacy LUMC, Leiden, The Netherlands) and stored at –20°C. Endotoxin concentrations were below detection level.

Cell Culture

Peripheral blood mononuclear cells were isolated from buffy coats of healthy donors using Ficoll-amidotrizoate density gradient centrifugation. Monocytes were further purified by CD14-positive selection using anti-CD14-conjugated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer’s protocol. Next, monocytes (>95% pure, viability >96% as determined by propidium iodide staining) were incubated for 7 days in culture medium (RPMI-1640, Gibco Invitrogen, Breda, The Netherlands) containing 10% heat-inactivated fetal bovine serum (Greiner Bio-One BV, Alphen a/d Rijn, The Netherlands), 2 mM penicillin, 2 mM streptomycin (both PAA GmbH, Pasching, Germany) and 2 mM L-glutamine (Gibco Invitrogen) supplemented with 10 ng/ml recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) and 10 ng/ml recombinant human IL-4 (both Biosource, Camarillo, Calif., USA). To study the effect of hLF1–11 on differentiation of monocytes into iDCs, at the start of the culture, monocytes were exposed to various concentrations of hLF1–11 (up to 100 μ g/ml), control peptide (100 μ g/ml) or saline. On day 6, the iDCs were matured with heat-killed (30 min at 100°C) *Candida albicans* (1×10^7 CFU/ml strain Y01–19, Pfizer Inc., Groton, Conn., USA) for 20 h. Thereafter, supernatants were harvested for assessment of cytokine (and chemokine) levels, or iDCs and mature DCs were harvested at day 7 and used for analysis of their endocytic and phagocytic activities and their ability to produce reactive oxygen species (ROS) and to polarize CD4+ T cell differentiation in co-culture experiments.

Flow Cytometric Analysis of Cell Surface Molecule Expression by DCs

For measurements of the expression of a variety of cell surface molecules, the following monoclonal antibodies were used: phycoerythrin (PE)-conjugated antibodies directed against CD11b, CD40, CD54, CD80, CD83 and CD86 and fluorescein isothiocyanate (FITC)-labeled antibodies against HLA-DR, CD14, CD206 and CD209 were all obtained from BD Biosciences (Heidelberg, Germany). PE-conjugated antibody against dectin-1 was purchased from R&D Systems and Alexa Fluor 647-conjugated antibody against CD197 from BD Biosciences. DCs were harvested and resuspended in ice-cold 0.2% PBS/BSA, washed twice and then incubated with the selected antibodies for 30 min on ice in the dark. Cell surface molecule expression was assessed on a FACSCalibur and analyzed by BD CellQuest software (BD Biosciences). Results are expressed as median fluorescence intensity (MFI) corrected for background measurements.

Flow-Cytometric Analysis of Dextran-FITC Uptake by DCs

The endocytic property of the various iDCs was examined by incubation of the cells with 1 mg/ml FITC-labeled dextran (Invitrogen) for 2 h at 37°C in culture medium. Background measurements were performed at 4°C. After washing, dextran-FITC fluorescence was assessed using a FACSCalibur and analyzed by BD CellQuest software (BD Biosciences). The results are expressed as MFI corrected for background measurements.

Assays for the Phagocytosis of *Staphylococcus aureus* and *C. albicans* by Immature DCs

Phagocytosis of pHrodo-labeled *S. aureus* was performed as described for macrophages [6]. Briefly, a stock suspension of pHrodo-labeled *S. aureus* (Invitrogen) was prepared according to manufacturer's protocol. pHrodo is a dye that is non-fluorescent at neutral pH and bright red in acidic environments (e.g. phagolysosome). Equal volumes of iDCs (1×10^6 /ml) and 5 times prediluted pHrodo-labeled *S. aureus* stock were mixed and then incubated for several intervals of time at 37°C, or as a control, at 4°C. Thereafter, pHrodo fluorescence of the iDCs was assessed on a FACSCalibur. Results are expressed as the percentage of pHrodo-positive iDCs.

Phagocytosis of *C. albicans* by iDCs was assessed by FACS analysis as described for macrophages [6]. In short, *C. albicans* cultured overnight were washed twice in PBS and then labeled with 0.5 μ M carboxyfluorescein succinimidyl ester (CFSE, Invitrogen) for 30 min at 37°C in the dark, centrifuged and resuspended in RPMI-1640 supplemented with 20% human serum. iDCs were washed twice in PBS/0.2% bovine serum albumin (BSA) and then labeled with PE-conjugated antibody against CD54 for 30 min (on ice in the dark). Next, these labeled iDCs were mixed with CFSE-labeled *C. albicans* in a 1:1 ratio and incubated for various intervals at 37°C under slow rotation in the dark. The percentage of iDCs associated with *C. albicans* was assessed by determining the percentage of double-positive iDCs (CD54+/CFSE+) using a FACSCalibur and analyzed by BD CellQuest software. Control experiments were performed at 4°C to correct for binding of *C. albicans* to iDCs. Results are expressed as percentage iDCs that were positive for both CD54 and CFSE.

Flow Cytometric Analysis of ROS Production by DCs

Intracellular ROS production by iDCs was quantified using the fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA) (Invitrogen). In short, iDCs were loaded with 10 μ M DCFH-DA for 20 min at 37°C in the dark. Thereafter, iDCs were incubated with 1×10^7 heat-killed *C. albicans* for various intervals of time. ROS production was measured on a FACSCalibur. The results are expressed as MFI with interquartile range.

Determination of Cytokine Levels by ELISA

Enzyme-linked immunosorbent assay (ELISA) cytosets were used to determine the concentrations of interleukin (IL)-6, IL-10, IL-12p40 and TNF- α (Invitrogen) according to manufacturer's instructions.

T Cell Isolation and DC-T Cell Cocultures

CD4⁺ T cells (>95% purity) were obtained from the CD14-negative fraction after monocyte isolation (stored at -80°C) by CD4-positive selection using anti-CD4-conjugated magnetic microbeads (Miltenyi Biotec) according to the manufacturer's pro-

cedure. They were resuspended in Iscove's Modified Dulbecco's Medium (Lonza, Verviers, Belgium) containing 10% heat-inactivated fetal bovine serum (Gibco), antibiotics and 2 mM L-glutamine and left overnight to recover. iDCs were reseeded in a 96-well plate (1×10^5 /well). Three hours thereafter, cells were stimulated with a Th17-favoring mix comprising of purified protein derivative of *Mycobacterium tuberculosis* (PPD, Statens Serum Institute, Copenhagen, Denmark; 5 μ g/ml) [19, 20], lipopolysaccharide (LPS, 100 ng/ml; Sigma-Aldrich, Zwijndrecht, The Netherlands), tetanus toxoid (TT; 1% of the stock, 80 IU/ml) [21, 22] and heat-killed *C. albicans* (1×10^6 /ml) [20, 22]. Twenty-four hours thereafter, DCs were cocultured with 1×10^6 autologous T cells for 72 h in the presence of TT (1% of the stock, 80 IU/ml) and supernatants were collected and assessed for IL-17, IL-10, IFN- γ , IL-2 and IL-4 levels using a custom-made multiplex bead-array (Bio-Rad, Hercules, Calif., USA), according to the manufacturer's protocol. For intracellular cytokine staining, cells in coculture were incubated with brefeldin A (3 μ g/ml, Sigma) during the last 18 h of coculture, replicates ($n = 10$) were pooled and T cells were labeled extracellularly using anti-CD3-AMCyan, anti-CD4-PECy7 and anti-CD25-FITC (all BD Biosciences) antibodies. Next, cells were fixed and intracellularly labeled using Intrastain reagents (DakoCytomation, Heverlee, Belgium) with PE-labeled antibodies directed against IL-17 (Ebioscience, San Diego, Calif., USA), Alexa 700-conjugated antibodies against IFN- γ (BD Biosciences) and allophycocyanin (APC)-labeled antibodies against IL-10 (Miltenyi Biotec) before acquisition on a LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software version 8.7.3 (Tree Star Inc., Ashland, Oreg., USA). The core population of live CD3⁺ T cells was analyzed for IL-17 production while activated and CD3^{dim} T cells were also included in IFN- γ production analysis.

Statistical Analysis

Friedman followed by Dunn's multiple-comparisons post-hoc test or, where indicated, Wilcoxon's test was used to determine the differences between the results for hLF1-11-differentiated and control (peptide-incubated) DCs. Data are expressed as median and range. Two-sided p values are reported and the level of significance was set at $p < 0.05$.

Results

Morphology and Cell Surface Molecule Expression by hLF1-11-Differentiated and Control (Peptide-Incubated) iDCs and Mature DCs

First, we inspected the morphology of the DCs that had been differentiated in the presence of hLF1-11 or the control peptide. The morphology, density and attachment of the cells to the wells did not differ between hLF1-11 and control (peptide-incubated) DCs. Next, we compared cell surface molecule expression by hLF1-11-differentiated and control (peptide-incubated) iDCs as an easy read-out for possible hLF1-11-induced phenotypic differences by these DCs. The results revealed that

Table 1. Cell surface molecule expression by hLF1-11 and control (peptide-differentiated) DCs

		Immature DCs			<i>C. albicans</i> -matured DCs		
		control	hLF1-11	control peptide	control	hLF1-11	control peptide
CD14	LPS coreceptor	2 (1-3)	4 (2-7)*	2 (1-3)	3 (2-7)	2 (1-6)	2 (0-6)
CD83	Maturationmarker	1 (0-4)	2 (0-3)	1 (0-3)	32 (21-54)	22 (12-42)*	36 (26-74)
CD197	CCR7	8 (5-17)	12 (7-19)	10 (7-16)	29 (26-34)	27 (21-42)	32 (24-38)
CD54	ICAM-1	199 (123-241)	138 (92-245)	183 (109-256)	881 (512-1,190)	1,025 (500-1,434)	850 (356-1,155)
CD40	Costimulatory	52 (30-81)	57 (31-89)	56 (20-68)	86 (52-127)	77 (42-127)	70 (26-104)
CD80	B7.1	5 (0-9)	7 (0-19)*	4 (0-7)	32 (24-83)	29 (18-90)	30 (29-99)
CD86	B7.2	14 (0-26)	7 (2-48)	11 (4-25)	407 (233-459)	247 (217-375)*	382 (317-466)
HLA-DR	MHC class II	46 (24-100)	63 (36-252)*	45 (21-73)	170 (97-399)	138 (86-517)	147 (68-365)
CD11b	CRIII	294 (131-382)	261 (104-299)	288 (201-379)	278 (183-320)	224 (189-304)	269 (226-396)
CD206	Mannose receptor	45 (32-71)	33 (22-152)	54 (27-75)	30 (17-45)	31 (16-44)	33 (19-53)
CD209	DC-SIGN	44 (29-66)	44 (19-74)	42 (23-54)	41 (23-49)	41 (26-51)	33 (23-50)
Dectin-1	β -Glucan receptor	13 (9-23)	19 (13-39)*	12 (7-19)	20 (10-25)	19 (11-28)	21 (14-34)

Results are expressed as MFI from which background fluorescence by unlabeled cells was subtracted from the values for labeled cells. Data are expressed as medians and ranges (in parentheses) in experiments with at least 6 different donors. * $p < 0.05$.

hLF1-11-differentiated iDCs expressed significantly higher levels of HLA class II and dectin-1, a receptor involved in *C. albicans* recognition, on their cell-surface as compared to control (peptide-incubated) iDCs on their surface. In addition, expression of CD14 and CD80 was marginally enhanced (table 1). Upon maturation by *C. albicans*, the expression of the maturation marker CD83 as well as the costimulatory molecule CD86 by hLF1-11-differentiated DCs was significantly decreased as compared to control (peptide-incubated) DCs (table 1).

Endocytic and Phagocytic Properties of hLF1-11-Differentiated and Control (Peptide-Incubated) DCs

Since hLF1-11-differentiated DCs seemed to be affected by hLF1-11 treatment during differentiation, we compared the endocytic and phagocytic capacities of iDCs differentiated in the presence of hLF1-11 with those of control (peptide-incubated) iDCs. No significant difference in dextran uptake between hLF1-11-differentiated iDCs and control (peptide-incubated) iDCs was observed (fig. 1a). In addition, phagocytosis of *S. aureus* by hLF1-11-differentiated iDCs did not differ from that by control (peptide-incubated) iDCs (fig. 1b). However, the percentage of *C. albicans*-phagocytosing hLF1-11-differentiated iDCs was significantly higher than that of control (peptide-incubated) iDCs (fig. 1c). Control experiments at 4°C revealed that approximately 10% of the iDCs bound *C. albicans* at all intervals of time, independent of the presence of hLF1-11 or control peptide during differentiation (data not shown).

ROS Production by hLF1-11-Differentiated and Control (Peptide-Incubated) iDCs in Response to *C. albicans*

Since hLF1-11-differentiated iDCs displayed enhanced phagocytosis of *C. albicans*, we next determined the production of ROS by the various iDCs in response to this yeast. Results revealed that hLF1-11-differentiated iDCs produced significantly more ROS 30 and 60 min after stimulation with *C. albicans* than control (peptide-incubated) iDCs (fig. 2).

Differential Cytokine Production Patterns in hLF1-11-Differentiated DCs and Control (Peptide-Incubated) DCs

Another functional property of DCs is the production of inflammatory mediators. We therefore assessed the pro- and anti-inflammatory cytokine production by the various DCs in response to *C. albicans*. The results showed that heat-killed *C. albicans*-matured hLF1-11-differentiated DCs produced significantly more IL-6 and IL-10 and less IL-12p40 than control (peptide-incubated) DCs (fig. 3). The production of TNF- α did not differ between the various groups of DCs (fig. 3).

Polarization of CD4⁺ T Cells by hLF1-11-Differentiated or Control (Peptide-Incubated) DCs

Next, we compared the ability of hLF1-11-differentiated DCs to direct T cell polarization and that of control (peptide-incubated) DCs using a Th17 favoring mix. The supernatants of the cocultures of hLF1-11-differentiated

Fig. 1. Endocytosis of FITC-dextran and uptake of *C. albicans* and *S. aureus* by hLF1-11-differentiated and control (peptide-incubated) iDCs. Monocytes were cultured with rhGM-CSF and IL-4 in the presence of hLF1-11 (100 μ g/ml, dark gray bars), control peptide (CP; 100 μ g/ml, light gray bars) or no peptide (open bars). At day 7, iDCs were harvested, washed and cultured for 2 h in the presence of FITC-dextran; thereafter the amount of dextran uptake by these cells was assessed by flow cytometry (a). Furthermore, after 60, 90 or 120 min coincubation of the iDCs with pHrodo-labeled *S. aureus*, the percentage of pHrodo-positive iDCs was determined by flow cytometry (b). Lastly, iDCs were coincubated with CFSE-labeled *C. albicans* and after 15, 30 and 60 min coincubation, the percentage of iDCs associated with *C. albicans* was determined by flow cytometry (c). Boxes represent medians and second and third interquartiles, whiskers represent ranges in experiments with 6–10 different donors. * $p < 0.05$.

Fig. 2. Intracellular ROS production by hLF1-11-differentiated and control (peptide-incubated) iDCs. Monocytes were cultured with rhGM-CSF and IL-4 in the presence of hLF1-11 (100 μ g/ml, dark gray bars), control peptide (100 μ g/ml, light gray bars) or no peptide (open bars) and labeled DCFH-DA at day 7 with. Next, the labeled iDCs were stimulated with heat-killed *C. albicans* and the MFI was assessed directly and after 30, 60 and 90 min as a measure of ROS production. Boxes represent medians and second and third interquartiles, whiskers represent ranges in experiments with 8 different donors. * $p < 0.05$; ** $p < 0.01$.

Fig. 3. Cytokine profiles of hLF1-11-differentiated and control (peptide-incubated) DCs in response to heat-killed *C. albicans*. Monocytes were cultured with rhGM-CSF and IL-4 in the presence of hLF1-11 (1, 10, 100 μ g/ml; dark gray bars) or control peptide (CP, 100 μ g/ml; light gray bars) for 6 days. Thereafter, cells were stimulated with 1×10^7 heat-killed *C. albicans* for 20 h and then supernatants were collected and assessed for IL-6 (a), IL-10

(b), IL-12p40 (c) and TNF- α (d) levels. Values are expressed as fold increase of cytokine production compared to control DCs (none, no peptide; open bars). Data are expressed as boxes and whiskers; boxes represent medians and second and third interquartiles, whiskers represent ranges in experiments with 7 or 8 different donors. * $p < 0.05$; ** $p < 0.01$ as compared to control and control peptide-incubated DCs.

DCs with autologous CD4 $^+$ T cells contained significantly more IL-17 ($p < 0.05$) and IL-10 ($p = 0.06$), but less IFN- γ (fig. 4a) than the supernatants of cocultures with control (peptide-incubated) DCs. In these cultures, IL-2 was out of range and no IL-4 was detected (data not shown). In addition, we determined the percentage of T-cells expressing the various cytokines (IL-17, IL-10 and IFN- γ) by intracellular cytokine staining. Results revealed that enhanced percentages of IL-17-producing T cells and reduced percentages of IFN- γ -producing T cells were found in the cocultures with hLF1-11-differentiated DCs as compared to control (peptide-incubated) DCs (fig. 4b). IL-10-producing T cells were present at very low frequencies and no differences in percentages of IL-10-producing T cells were found between the different groups (fig. 4b), suggesting that IL-10 in the supernatants of the coculture (fig. 4a) is mainly derived from the DCs.

Discussion

In this study, we demonstrated that the presence of hLF1-11 during differentiation of monocytes into DCs results in DCs that promote antifungal responses to *C. albicans* and Th17 polarization. This conclusion is based on the following findings. First, phagocytosis of *C. albicans* and ROS production in response to this yeast were elevated in hLF1-11-differentiated DCs as compared to the control (peptide-incubated) DCs. Surprisingly, phagocytosis of *S. aureus* by hLF1-11-differentiated DCs was not enhanced. Second, the production of IL-6 and IL-10 by hLF1-11-differentiated DCs in response to *C. albicans* was significantly enhanced, while IL-12p40 production was reduced and TNF- α expression unaffected as compared to control (peptide-incubated) DCs. Incubation of hLF1-11-differentiated DCs with bacterial stimuli like

Fig. 4. Cytokine profile by CD4⁺ T cells after coculture with hLF1-11-differentiated and control (peptide-incubated) DCs. Monocytes were cultured with rhGM-CSF and IL-4 in the presence of hLF1-11 (100 µg/ml; dark gray bars), control peptide (CP, 100 µg/ml; light gray bars), or saline (open bars) for 6 days. Thereafter, cells were stimulated with heat-killed *C. albicans* (5×10^5 /ml), purified protein derivative of *M. tuberculosis* (PPD; 5 µg/ml), TT; 150 lf/ml) and LPS (100 ng/ml) for 24 h. Next, cells were washed and CD4⁺ T cells from the same donor and TT were add-

ed to the culture. Seventy-two hours later, supernatants were harvested and assessed for IL-17, IL-10 and IFN-γ (**a**) and IL-4, IL-2 levels (data not shown). Next, intracellular cytokine production by the T-lymphocytes in the coculture was assessed by addition of brefeldin A for the last 16 h of the coculture (3 µg/ml). T cells were stained for intracellular IL-17, IL-10 and IFN-γ (**b**). Data are expressed as boxes and whiskers; boxes represent medians and second and third interquartiles, whiskers represent ranges in experiments with 6–8 different donors. * $p < 0.05$.

LPS or lipoteichoic acid resulted in a significant ($p < 0.05$) reduction of TNF-α and IL-12p40 (without affecting the IL-6 and IL-10 levels) as compared to control (peptide-incubated) DCs (online supplementary figure 1 and 2, www.karger.com/doi/10.1159/000332941). Together, these data indicate that the presence of hLF1-11 during mono-

cyte-DC differentiation has differential effects on the cytokine profile of the resulting DC upon maturation by fungal or bacterial stimulation. Interestingly, we reported earlier that the presence of hLF1-11 during GM-CSF-driven differentiation of monocytes into macrophages led to macrophages displaying enhanced production of IL-10, but not

IL-12p40 or TNF- α , in response to *C. albicans* [6]. In addition, these macrophages displayed enhanced antimicrobial properties against *C. albicans* as well as *S. aureus*. One possible explanation for the differential effects of hLF1-11 on monocyte-DC differentiation and monocyte-macrophage could involve myeloperoxidase – the major intracellular target of hLF1-11 [van der Does et al., submitted]. GM-CSF enhances the expression of myeloperoxidase in monocytes, thereby creating more ‘target’ for hLF1-11 for modulation, whereas GM-CSF and IL-4 (or IL-4 alone) diminish myeloperoxidase expression [23].

The most striking finding of this study is that hLF1-11 drives differentiation of monocytes toward DCs that enhance Th17 polarization. Th17 responses are thought to be important in host defense against fungi and *S. aureus* [24–27], especially in the skin and mucosa. In addition, IL-17 is involved in the influx of neutrophils and can induce production of cytokines/chemokines and antimicrobial peptides by epithelial cells [28].

Although Th17 cells are part of the adaptive immune response, they serve mainly to regulate innate immune responses [29]. Currently, the mechanism(s) underlying the enhanced Th17 polarization by hLF1-11-differentiated DCs are not clear. However, Bouguermouh et al. [30] have shown that mature DCs are less capable of inducing Th17 polarization than iDCs due to reduced stimulation of CD28 on the T cells by CD80 and/or CD86. hLF1-11-differentiated DCs displayed reduced maturation as indicated by reduced CD83 expression as well as CD86 expression.

In addition to enhancing Th17 polarization, hLF1-11-differentiated DCs reduced the IFN- γ production by CD4+ T cells. It cannot be excluded that this reduction of Th1 polarization is the consequence of the enhanced IL-10 production by the hLF1-11-differentiated DCs in the cocultures [31].

Interestingly, the effects of hLF1-11 on monocyte-DC differentiation differ from those reported for other AMPs/HDPs, such as the human cathelicidin LL-37 and

α - and β -defensins. Davidson et al. [14] showed that the presence of LL-37 during monocyte-DC differentiation resulted in DCs promoting IFN- γ -producing T cells. Kandler et al. [16] have shown that this peptide inhibited the response of iDCs toward microbial stimuli such as LPS, thereby indicating that LL-37 reduced the maturation of these cells. Others reported that human defensins HNP-1 and hBD-1 promoted the activation and maturation of DCs and stimulated the production of TNF- α , IL-6, and IL-12p70 but not IL-10 [32]. Together, hLF1-11 and LL-37/defensins differentially affect the differentiation and subsequent maturation of DCs and their ability to promote Th cell polarization.

The concentration of hLF1-11 in the present study (100 μ g/ml) was determined based on dose-response experiments with monocytes, in earlier experiments [6, 18]. In addition, hLF1-11 has been shown to be effective in mice up to concentrations of 4 mg/kg body weight, which corresponds to injection of 100 μ g/mouse. However, it should be realized that extrapolation of in vitro to in vivo levels is hampered as the local concentration of the peptide at the site of infection remains unknown.

Together, these data show that hLF1-11 is able to modulate monocyte-DC differentiation, resulting in DCs displaying enhanced antifungal responses against *C. albicans* and the ability to promote Th17 polarization after coculture with CD4+ T cells. The immunomodulatory properties of hLF1-11 may aid in balancing the immune responses of the host, leading to the resolution of infections by (multidrug)-resistant pathogens.

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